A brief overview of intracortical circuits

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Abstract: This report aims at giving an insight of the most salient features of intracortical connectivity, i.e., the structure of neuronal networks inside a cortical area. In the first part we raise the question of cortical columns, a blur and sometimes misused concept defining fundamental mesoscopic units in the cortex. Their role and structure show a lot of discrepancies across species and even across areas. In the second part, we focus on local circuits and try to get an insight of their complexity as well as their most important organizing laws, like the stereotypical excitatory pathway. The last part is dedicated to horizontal connectivity, illustrated through two famous examples: the primary visual cortex of mammals and the rat barrel cortex.

Key-words: cortex, neurons, cortical areas, cortical layers, cortical columns, macrocolumn, microcolumn, microcircuits, connectivity, excitatory neurons, excitatory pathway, inhibitory neurons, interneurones, neuronal compartments, selectivity, primary visual cortex, barrel cortex, horizontal connectivity, patchy connectivity

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Introduction aux circuits intracorticaux

Résumé : Ce rapport présente plusieurs aspects de l'organisation intracorticale, c'est-à-dire l'organisation du réseau de neurones au sein d'une aire corticale. La première partie traite la question des colonnes corticales, des unités mésoscopiques fondamentales difficiles à cerner, dont le role et la structure varie d'une espèce et même d'une aire à l'autre. Dans la deuxième partie, nous montrons la grande complexité des circuits corticaux locaux tout en essayant d'en extraire des principes organisationnels et fonctionnels généraux, comme par exemple le trajet stéréotypé de l'excitation dans les différentes couches corticales. Enfin, la troisième partie est consacrée à la connectivité horizontale, illustrée à travers deux aires bien connues: le cortex visuel primaire des mammifères et le cortex sensoriel du rat.

Mots-clés : cortex, neurones, aires corticales, colonnes corticales, macrocolonne, microcolonne, microcircuits, connectivité, neurones excitateurs, circuit excitateur, neurones inhibiteurs, interneurones, compartiments neuronaux, sélectivité, cortex visuel primaire, tonneaux corticaux, connectivité horizontale

Contents

The cortex is the superficial part of the encephalon and represents the biggest part of grey matter in the brain. It has a horizontal organization in layers (*laminae*) of different types of cells (figure 1). The number of layers, their cell composition, their thickness and organization are not the same over the surface of the cortex. Those differences led neuroanatomists to divide the cortex into small regions called *areas* (figure 2) where those characteristics were homogeneous and that corresponded to different functions, e.g., vision or motion. Nevertheless most of the cortex is made up of six layers of neurons, from layer I at the surface of the cortex to layer VI that lies next to the white matter. For humans, its thickness varies from 3 to 6 mm.
About fourty types of neurons have been identified through the cortex but they can essentially be divided into only two classes: *projection neurons* and *local interneurons*. Projection neurons (also called *principal neurons*) are excitatory cells, most of them having a pyramidal cell body and being situated in layers III, V and VI of the cortex. Interneurons can be found in all layers but they just amount to 20 up to 25% of cortical neurons and are often inhibitory. Information processing in the cortex is multi-step and the axons of projection neurons carry information from one stage to the next, sometimes in distant groups of neurons. Interneurons can receive the same input as principal neurons but just convey it to local cells implied in the same stage of information processing. More detailed information about cortical structure and function can be found in [21, 37, 22].
The organization of the cortex is not only laminar. It has been observed that neurons one runs across perpendicular to the cortex tend to be connected to each other and to respond to precise stimulations with similar activities throughout the layers. They form a *cortical column*.

1 Biological cortical columns

1.1 The anatomical column

Many cortical neurons throw their axons and dendrites from the cortex surface to the white matter thereby forming the anatomical basis of the columnar organization in the cortex (figure 3-B and 4). Nervous fibers from the thalamus mostly end in layer IV where they are connected to stellate neurons. These neurons throw their axons towards the surface of the cortex, parallel to apical dendrites of neighboring pyramidal neurons, and establish connections with them (figure 3-C). The thalamocortical input is therefore conducted within a thin column of strongly connected cells so that the same information is shared throughout the depth of the cortex perpendicular to its surface [22].
Several studies have shown biological evidences for such small aggregates of about one hundred neurons, 20 up to 50 μm wide, called *minicolumns* or *microcolumns*. Their formation is due to the radial migration of neurons during brain development [10, 34] (see figure 4).
However the minicolumn hypothesis does not solve the problem of defining cortical columns. They have not been extensively observed among species, nor among cortical areas. Moreover, horizontal connectivity should not be underestimated and neighboring minicolumns, far

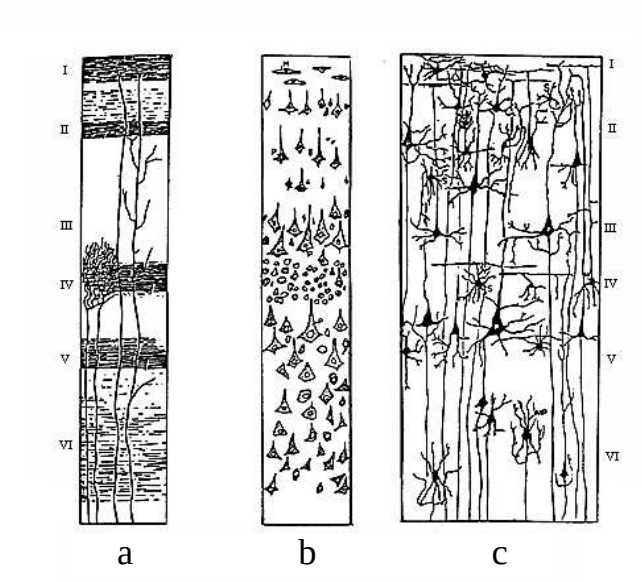

Figure 1: *Layer organization of the cortex (a) Weigert's coloration shows myelinated fibers (axons) and so the connections inside and between layers, (b) Nissl's coloration only reveals cell bodies (c) Golgi's coloration shows the whole cells (From [35]).*

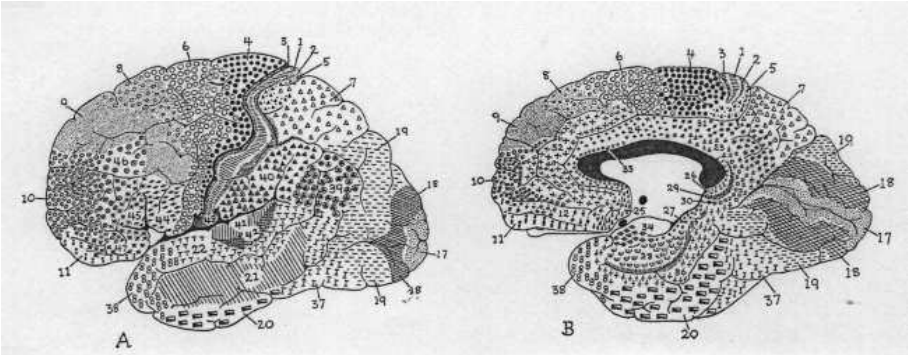

Figure 2: *In 1909, Brodmann [9] divided the cortex into 52 cytoarchitectonic areas according to the thickness of the cortical layers. For example, layer IV is very thin in the primary motor cortex (area 4) while it is very thick in the primary visual cortex (area 17).*

from being isolated, make numerous connections. These horizontal connections explain the observation of larger columnar units.

1.2 Cortical columns as physiological units

In 1957, Mountcastle discovered a columnar organization in the cortex [33] (see figure 5). With electrode recordings, he showed that neurons inside columns of 300 to 500 μm of diameter displayed similar activities. Those physiological units are usually called *macrocolumns*. In figure 6, we see physiological columns obtained from the diffusion of a radioactive substance.
Some of them are spatially well defined while some ohers are more difficult to distinguish from one another. What is the meaning of such units?

1.3 From physiological to functional units

Many experiments on somatosensory and visual cortices made it possible to relate physiological columns with sensory functions [22, 33, 20, 19, 29] (see figure 6, 7 and 8). In some cases the processing site of a given function seems quite clearly defined like in rat's sensory cortex where every whisker is associated with a sharply bounded cortical site in layer IV (see figure 7). However such a *barrel* structure is less evident in other layers so that it is harder to distinguish columns and label them with a given whisker.
Although, this particular case shed light on the important role afferent thalamic fibers play in shaping columns in sensory cortices [29]. These axonal fibers project in layer IV in the form of tufts spanning horizontally over a few hundreds of micrometers, therefore exciting and, up to horizontal connectivity, defining the width of physiological columns processing

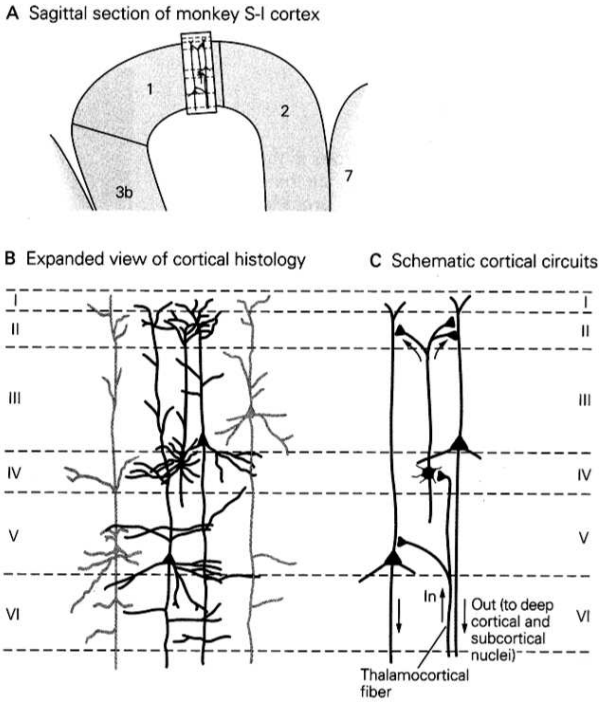

Figure 3: *(A) Sagittal section of the primary somatosensory cortex of the monkey (S-I) (B) Morphology of relay cells from layers III to V. Stellate neurons (layer IV) receive information from the thalamus and transmit it to neighboring pyramidal cells in superficial layers of the cortex. Pyramidal cells throw their axons towards deep layers of the cortex and other cortical or sub-cortical regions. They also establish horizontal connections with neighboring columns sharing the same physiological properties (C) Diagram of intra-cortical excitatory circuitry (From [22]).*

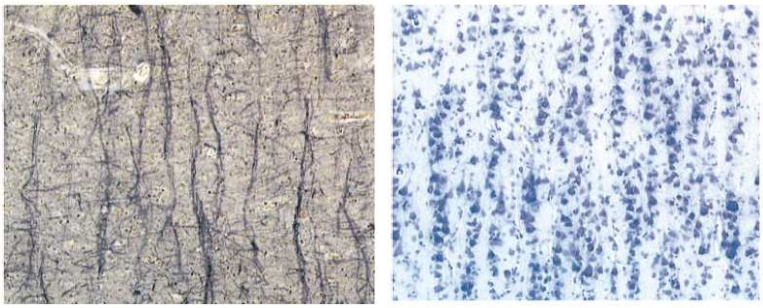

Figure 4: *Myelinated fibre bundles (left) and cell soma aggregates (right) in the same cortical region. These observation suggest minicolumnar organization in the cortex(From [10]).*

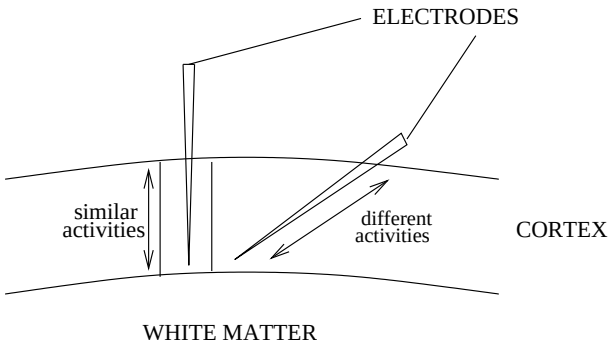

Figure 5: *Mouncastle's pioneering experiment. When he moved an electrode perpendicular to the cortex surface, he encountered neurons with similar electrical activities while moving the electrode obliquely gave him different types of recordings. So he showed the existence of 300-500 μm wide columns in the cortex.*

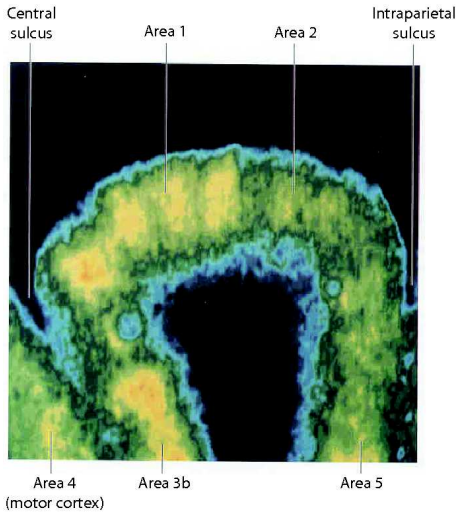

Figure 6: *Columns from the primary somatosensory cortex shown by auto-radiography after 45 minutes of stroking the hand of a monkey with a brush. On this sagittal section of the cortex a high activity (proportional to the concentration of a radioactive substance) can be viewed in areas 1 and 3b. Columns are well defined in area 1 but not in area 3b (From [22]).*

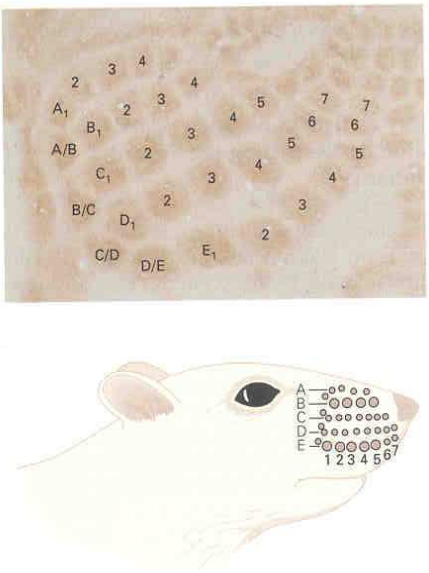

Figure 7: *Layer IV of rat's sensory cortex (stained for serotonin). Every whisker of the rat corresponds to a well defined area of the cortex mostly responsible for processing information from it. These processing units have the same distribution as whiskers on the muzzle. (From [22]).*

input for a given sensory function. Actually, the picture is more complex since different thalamic outputs may project on the same cortical column and one thalamic output may project on different cortical columns.
Anyway, functional columns usually overlap if the corresponding functions are close and information processing columnar sites move continuously across the surface of the cortex as function "continuously" varies, as it is observed in somatotopy, retinotopy, or orientation preference experiments (figure 8-B, left image, and figure 9, left image) [20, 19, 16, 36]. Nevertheless, functional columns may have partial well-defined boundaries at the discontinuities of the functional map. Examples of these discontinuities are given, in the visual cortex, by pinwheel points in V1 or reversal direction lines in area 18 (V2), where functional columns are sharply separated (see figure 8 and 9, right image).
To finish our discussion on functional columns, it is to be noted that these structures have not been observed in all regions of mammalians cortex and show species dependency. For example, there is no orientation preference columnar structure in rat primary visual cortex, which means that locally, cells with all orientation preference are represented. One usually speaks of it as a "salt and pepper" structure. This has been illustrated in [36] (see figure 10).

1.4 Who drives the cortical column activity?

We now raise the question of the driving forces that shape the activity of a cortical column. What is the respective influence of intrinsic columnar organization, thalamic input and neighbouring columns?
In [30], the authors use calcium imaging to observe the activity of neurons in *in vitro* thalamo-cortical slices of a few millimeters. They investigated the relation between spontaneously generated cortical activity and the activity generated by thalamic stimulation. A very good match have been found between spontaneous and triggered activities, both in the set of activated cells and in the temporal sequence of their activation (figure 11).
These results suggest that the thalamic input would just serve to awaken circuit dynamics that are intrinsic to the cortex. So, intracortical connectivity would play the primary role in determining the cortical response to thalamic input.

However, the intensity of the thalamic input to a column has a strong impact on the cortical response. We illustrate this with an optical imaging experiment in the rat barrel cortex (figure 12) [39]. A single whisker of a rat is deflected with different angles and speed. Because of the structure of the rat barrel cortex, this stimulus provokes a very localize input to the cortical barrel corresponding to the excited whisker, in layer IV. The experiment show that for weak stimulation, only one column will be activated: the one corresponding to the whisker. As the stimulus intensity increases, the activation is not anymore restricted to one column but spread horizontally to neighbouring columns. When the stimulation is strong enough, the whole barrel cortex is activated.
In addition to the influence of thalamic input on cortical response, this experiment shows that an excited column can activate its neighbours without the help of a thalamic stimu-

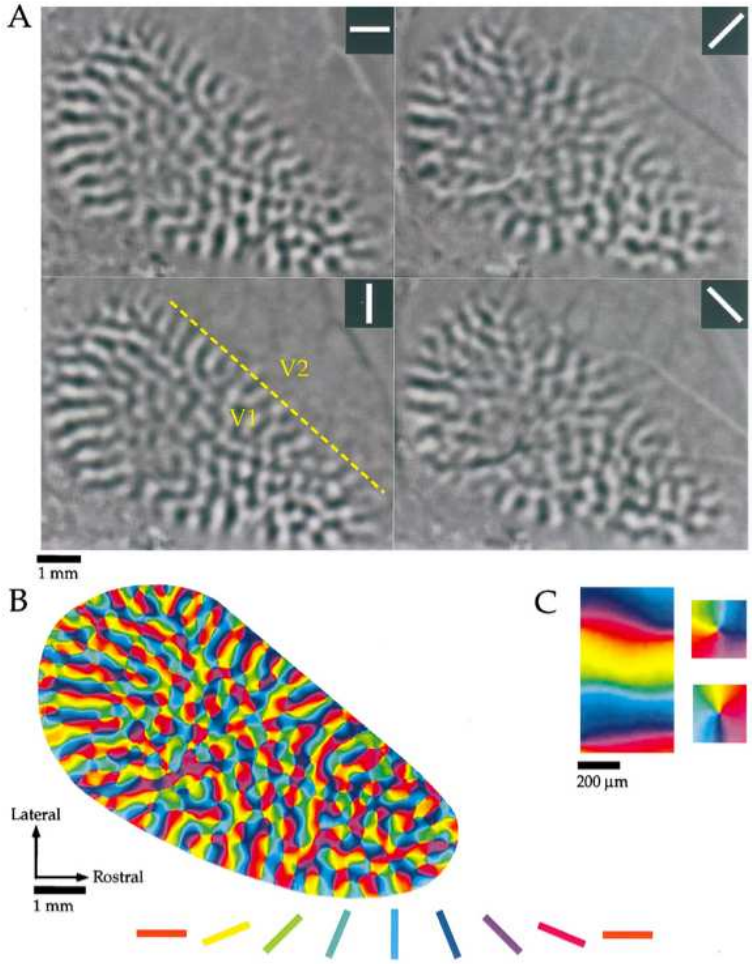

Figure 8: *Optical imaging of intrinsic signals in tree shrew visual cortex. A, Difference images obtained for four stimulus angles. Dark signal indicates areas that were active during presentation of the stimulus. B, Orientation preference map. Orientation preference of each location is color-coded according to the key shown below the map. C, Portions of the orientation preference map shown in B have been enlarged to demonstrate that the orientation preference maps contained both linear zones (left), and pinwheel arrangements (right) that are functional discontinuities (From [6]).*

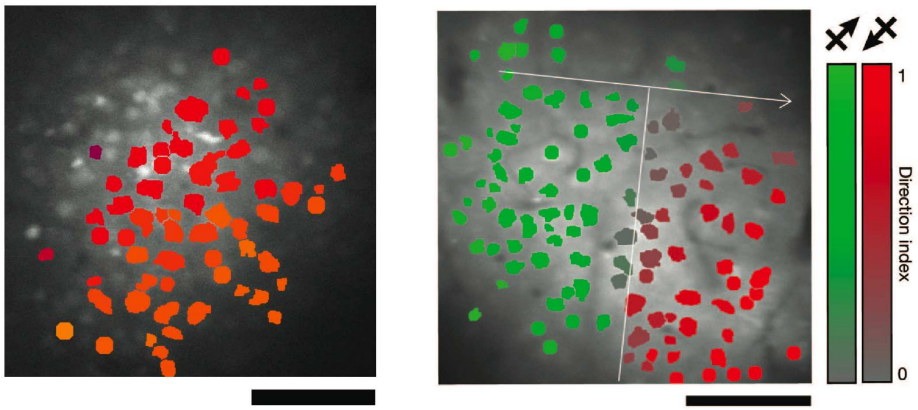

Figure 9: *Functional maps of selective responses in superficial layers of cat area 18 with single-cell resolution obtained by calcium imaging. Left: spatially smooth direction selectivity of neurons responding to a moving bar stimulus (preferred direction of movement:* 45° *for purple cells,* 90° *for red cells and* 135° *for orange cells). Right: discontinuity of direction selectivity giving partial boundaries to direction columns (From [36]).*

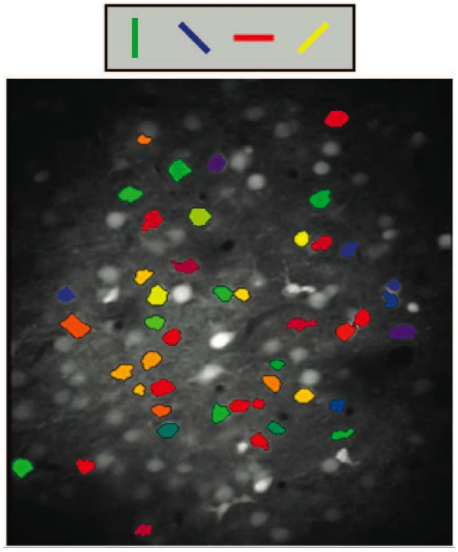

Figure 10: *Non-columnar orientation preference organization of rat primary visual cortex. Cells with different orientation preferences (see color code) are locally mixed together (From [36]).*

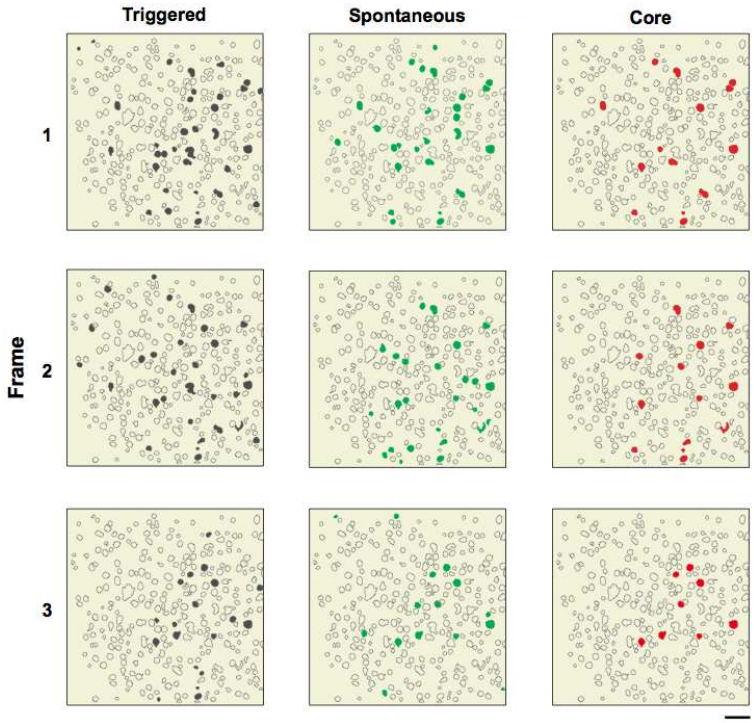

Figure 11: *Spatiotemporal dynamics of activation in spontaneous and triggered activity. Cells activated in the same order (frames 1, 2 and 3), over several activations. The three frames on the left show an example of spatiotemporal activation of the network when triggered by thalamic input (activated cells in gray). The three central frames correspond to spontaneous activation (green). Core frames indicate cells active in the same order across all movies from this slice. Scale bar, 50μm. (From [30]).*

lation of these. Hence a column can be a major driving force for the activity of its neighbours.

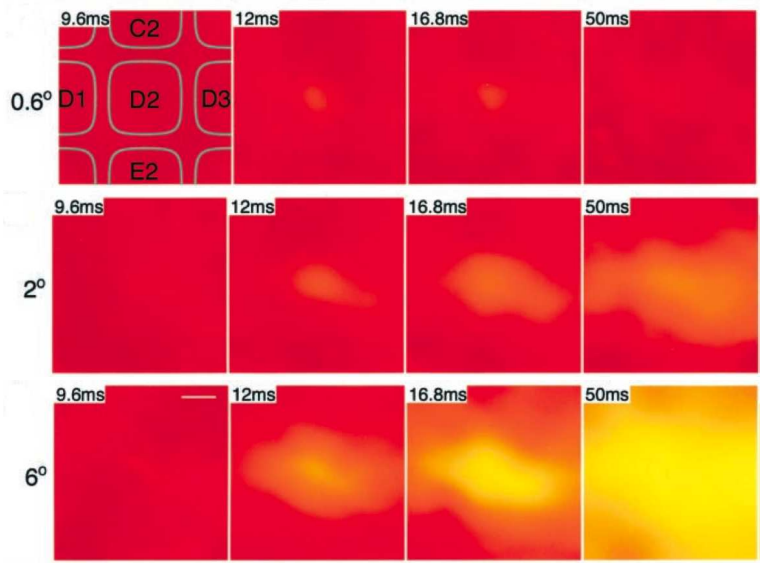

Figure 12: *Response sequences in the rat barrel cortex observed by optical imaging. A single whisker of the animal is stimulated with an increasing angle and speed (top to bottom). In the upper left image, a map of the barrels in layer IV is superimposed. A weak stimulation of the whisker provoke an activation in the column of barrel D2 (upper sequence). Stronger stimulation provoke graded horizontal spread of activity (middle and bottom sequences) (From [39]).*

A recent study has shed light on the multi-scale nature of columnar organization in the cortex by showing that functional columns are composed of finer scale, partially segregated, minicolumnar subnetworks [58]. So there are many ways of speaking of a cortical column. They form together a complex and sometimes blur or misused concept.

Now we try to understand the organization of columns by exploring cortical microcircuitry.

2 Local connectivity in the cortex

Local connectivity studies have been conducted on many species, in different areas of their cortices, with various theoretical and experimental tools. Although, most of them concern sensitive cortices (especially the primary visual cortex, V1) of mammalians (monkey, cat,

rat, human...), which have shown lots of similarities in composition, local and global structure. Here we will try to synthetize the main results of those studies. We want to get a good description of local connectivity inside a cortical area, like V1. So, we concentrate on connections between neurons in the same area, passing through gray matter (intracortical connections). Afferent and efferent connections coming from and going to other areas or thalamus, via white matter, do not belong to microcircuitry. Such connections will be thoroughly set apart from intracortical connections when we will have to mention them.
Cortical microcircuits show stereotypy, i.e. structural and functional repeating patterns, through lots of their features like cell types, cell arrangements or patterns of synaptic connectivity [42]. As long as considering a given area, age and species, it is reasonable to think of the cortical tissue as repeating a common local template with subtle variations. Yet microcircuits cannot be known with cell-by-cell precision across an area, stereotypy makes it possible to speak of an average connectivity pattern revealed by statistic connectivity studies.

2.1 Neuronal compartments and selectivity of synapses

Neurons are composed of several compartments: a cell body (*soma*), dendritic trees, and axonal branches (see figure 13 and 14). Usually the whole cell crosses several layers of the cortex, but neurons are commonly said to belong to a specific layer: the one their soma belongs to. However, this tells nothing on the location of the neuron's dendritic trees or axon terminals. For example, deep layers pyramidal cells have apical dendrites in the superficial layers of the cortex (see figure 14). So two neurons may contact in a layer none of their cell bodies belong to.
Excitatory cells (pyramidal cells: PC and spiny stellate cells: SSC) axons target dendrites of post-synaptic cells. Inhibitory cells axons have various target compartments on post-synaptic cells. Chandelier cells almost exclusively target the axon hillocks of pyramidal neurons. Large, small and nest basket cells provide most of the somatic and perisomatic inhibitory synapses for excitatory and inhibitory neurons. Dendrites are preferentially targeted by double bouquet, bitufted, bipolar, neurogliaform and Martinotti cells, with some types favoring either spines or shafts.
Several teams have recorded a huge amount of data on neuron, axon, dendrite and synapse density in the cortex of rodents and cat, by different techniques including 2D microscopy and 3D reconstruction of neurons [7, 3, 4, 13, 14, 37, 38]. These studies have led to connectivity estimations based on the idea that neurons establish *non-specific* synaptic contacts. This means that neurons project their axonal boutons across the cortex and locally make synapses with all represented types of neurons, proportionally to the fraction of dendritic surface these locally provide (Peters' rule, used by [7] and [5]).
Several recent studies show that connections are not just spatially uniformly random, depending on the local density of boutons and spines but are highly *selective* [44, 2, 52, 51, 57, 54, 27]. Dendrites and axons properties of pyramidal and inhibitory cells strongly suggest this *specificity* in intracortical connections. Pyramidal axons follow linear trajectories and typically form en passant boutons. These straight paths suggest that pyramidal axons do

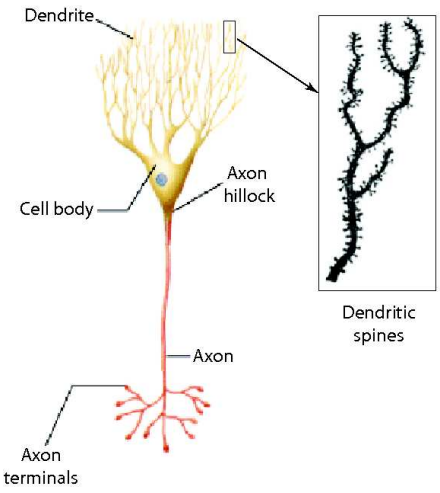

Figure 13: Neuronal compartments and dendritic spines.

not search out specific postsynaptic partners. On the other hand the dendrites of excitatory cells have large numbers of dendritic spines projecting up to 4 mm from the parent dendrite that are capable of movement and emerge at different angles from the dendritic shaft, suggesting that postsynaptic cells do seek out suitable excitatory inputs. Inhibitory neurones appear take the opposite approach with straight and largely spine free dendrites and axons that follow extremely complicated routes to make highly selective contacts with specific regions of their postsynaptic partners [2, 45, 47].
Connectivity studies taking specificity into account are mostly based on joint activity recordings, by calcium imaging or paired electrodes. Connections are established by physiological signals correlation and usually take the form of a connection probability between two types of neurons.
A quantitative description of interactions between each pair of neuronal types present in a local circuit has not yet been completed. However, some sub-networks are well known, like the excitatory pathway or certain intralaminar networks. The inhibitory network is way more complex, by the number of its neuronal types, their action and their role in the circuit.

2.2 Excitatory network

Excitatory cells account for approximately 70-80 % of neurons in the cortex and are divided into two main groups: pyramidal and spiny stellates. Both groups use glutamate as their primary neurotransmitter. PCs somata are situated in layers II to VI and SSCs situated within layer IV of primary sensory areas. The two classes have spiny dendrites. For that

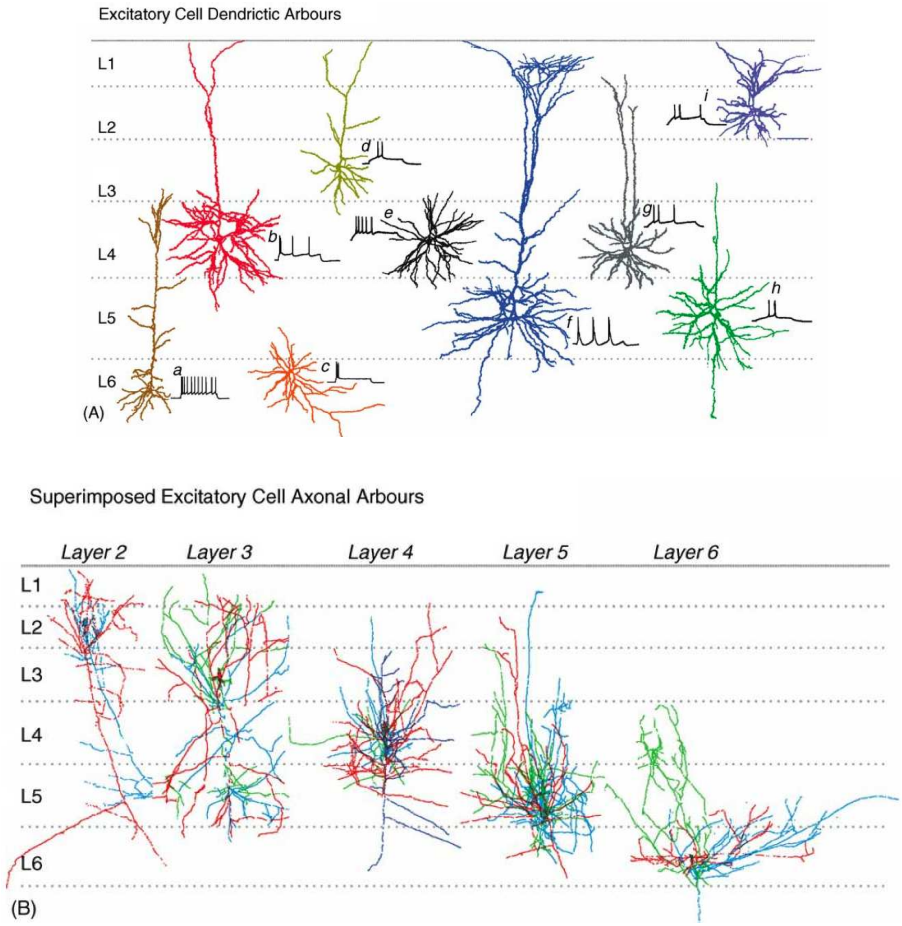

Figure 14: Dentritic and axonal trees of excitatory neurons (From [2]).

reason, excitatory cells in the cortex are often referred to as *spiny cells*. SSCs lack the long vertically oriented apical dendrite of PCs, so they do not get input from superficial layers. Moreover SSCs only establish local intracortical connections, while PCs project myelinated axons to the thalamus (cortico-thalamic projections) and other cortical areas (cortico-cortical projections). PCs show a lot of variability. While their anatomy is relatively stereotypical, their dendritic, axonal trees and spike discharge patterns are various among and inside layers [2].
The excitatory network is usually divided into two pathways. The feedforward pathway goes from layer IV (receiving cortical input from the thalamus) to layers II and III and then project to deep layers V and VI. It corresponds to the most basic and well-known trajectory of the thalamic input through cortical layers. The feedback pathway mainly consists in projections from layers V/VI to more superficial layers and from layer II/III to layer IV.

2.2.1 Layer IV

Layer IV is the main target of afferent thalamic fibers. These target both excitatory and inhibitory neurones in the highly interconnected networks in layer IV, but of all the synapses made with layer IV basket cells, thalamic inputs constitute only 13% and with SSCs only 6%. Yet, thalamocortical terminals are generally highly efficacious so that they reliably activate layer IV and sensory information from the thalamus strongly influences computations in the cortex. Moreover the majority of excitatory synaptic contacts to layer IV excitatory cells are supplied by other cells receiving direct input from the thalamus. Layer IV PCs and SSCs have extensive intralaminar axonal arbours, focussed projections to layer III and less dense projections to the deeper layers V and VI. So, they appear to have two major roles: amplify the input received in layer IV and feed excitation to layer III.

2.2.2 Layer II/III

Layer II pyramidal axons ramify most extensively in layers II and III where they make frequent connections with other local pyramidal cells. They also make long range projections through white matter. Layer III pyramidal axons ramify most extensively in layer II/III and V and make frequent synaptic connections with spiny cells there. Connections from layer III to V are highly selective. Layer III PCs mainly target large, tufted and intrisically bursting (IB) PCs in layer V and make very few connections to the smaller regular spiking (RS) cells. The rate of connectivity to IB cells is also dependent on the distance over which the two cells are separated laterally, the highest probability of connection being when the presynaptic cell was within $\sim 50\mu$m of the ascending post-synaptic apical dendrite. Layer III PCs also make back projections to layer IV with preferential innervation of inhibitory cells.

2.2.3 Layer V

The IB and RS pyramidal cells in layer V have similar patterns of axonal arborization. The axons of both classes ramify most extensively in layer V, indicating that they prefer to

innervate cells in their own layer since the pyramidal cells in more superficial layers do not have dendritic access to these dense arbours in layer V. Paired intracellular recordings reveal that while both classes are indeed synaptically connected, the RS cells are almost exclusively presynaptic to IB cells, which are in turn presynaptic to other IB cells. In addition to these dense intralaminar projections, layer V PCs project back to all other layers of the cortex, hence representing a major source of excitation to superficial laminae. Layer V PCs make very few and weak projections to layer III excitatory cells, but they do innervate inhibitory cells in this layer.

2.2.4 Back projections from PCs to layers III and V

These back projections mainly target inhibitory cells in the previous layer of the feedforward pathway. It suggests that pyramidal cells in each layer might, when generating outputs to integrated inputs, simultaneously initiate inhibition of their input layers via local circuit neurons (interneurones). This mechanism would be useful to prevent undesirable positive feedback loops and ensure the integrity of the excitatory flow through the cortex by silencing the cells responsible for their inputs as soon as appropriate outputs to the next stage of processing has been achieved [2].

2.2.5 Layer VI

Layer VI is the secondary main target of thalamic afferent fibers and also provides cortico-thalamic (CT) and cortico-cortical (CC) projections. PCs of layer VI that provide either CT or CC outputs can be identified by their distinctive morphologies. CT cells are typically oriented vertically, have short apical dendrites terminating in layer IV or in upper layer V and vertically oriented axonal arbours terminating in the vicinity of their dendrites. CC cells generate dendritic arbours that rarely extend beyond layer V and a characteristically long and horizontally oriented axon confined to the deep cortical layers. Phasically firing cells (those that respond to a sustained depolarising current pulse with a very brief train of action potentials followed by no further firing) would have CC-like morphology and tonically firing cells (those that fired continuous trains of action potentials for the duration of the depolarising pulse) would have CT-like morphology. The phasically firing CC-like cells principally innervate other layer VI PCs while tonically firing CT-like cells prefer to target interneurones. This specialization is not found in PCs subclasses of other layers. One possibility for the function of such selectivity in the connections of layer VI cells is that the CC cells may be responsible for conveying only the most novel information to other cortical regions via their phasic spike discharge. On the other hand, the CT cells which can maintain firing for longer, and prefer to activate interneurones, may therefore be responsible for the generation of powerful inhibition of the cortical columns in response to prolonged excitation both by thalamic afferents or by powerful local circuit connections [2].

2.3 Inhibitory action

20 to 30 % of cortical neurons are inhibitory cells. These cells use gamma-aminobutyric acid (GABA) as their primary neurotransmitter. Their relative density compared to excitatory cells or comparing the different types of inhibitory cells vary in different species, brain regions and layers. Most of these cells have aspiny dendrites, so that they are often termed as "smooth cells". Their axons usually arborize within a cortical column and can project laterally across cortical columns but do not typically project down into the white matter to contact the thalamus or distant brain regions, so they are also called "local circuit neurons" or "interneurones"[1].
The complexity of the inhibitory network and inhibitory action in the cortex has been demonstrated in several reviews [31, 53] and is the largest obstacle to unraveling the organization of cortical microcircuits. One of the main problem arises from the difficulty to define subclasses of inhibitory cells. Indeed, there are about ten well-known morphological types of interneurones (see figure 15) and a dozen of electrophysiological types. Each anatomical type has multiple firing patterns, which in turn correspond to multiple anatomical types of neurons. Hence there are more than fifty anatomo-electrophysiological types of interneurones in the cortex (see figure 16).
Interneurones can be roughly classified according to the post-synaptic neuronal compartment their axons preferentially innervate and have typical axonal arbors (see figure 15).

2.3.1 Main anatomical types of interneurones

- Basket cells (BC) represent 50% of inhibitory interneurones and are divided into three main subclasses on the basis of differences in dendritic and axonal morphologies. BCs are specialized in targetting the somata and proximal dendrites (i.e. those situated near the soma of a neuron) of their post-synaptic targets (PCs and interneurons). This position allow them to adjust the gain of integrated synaptic response and influence the action potential discharge, so that they play a role in phasing and synchronizing neuronal activity.
  - Large basket cells (LBC) provide lateral inhibition across columns in their layer of origin. They can also extend vertically, ramifying in several, often non adjacent layers. They have sparse axonal arbors with low terminal (also called "boutons") density on them. LBCs are largely represented in layers II-VI.
  - Small basket cells (SBC) have a very local impact since their axonal arbors are usually confined in the same column and layer as their soma. They have curvy frequently branching axons. They are present in layers II-VI, especially in layer IV.
  - Nest basket cells (NBC) have local axonal clusters and lower boutons density than LBCs. They are present in layers II-VI, and especially in layers II-IV.

[1] We will use the term interneurones to designate inhibitory interneurones, whereas there exist some classes of excitatory interneurones, like SSCs.

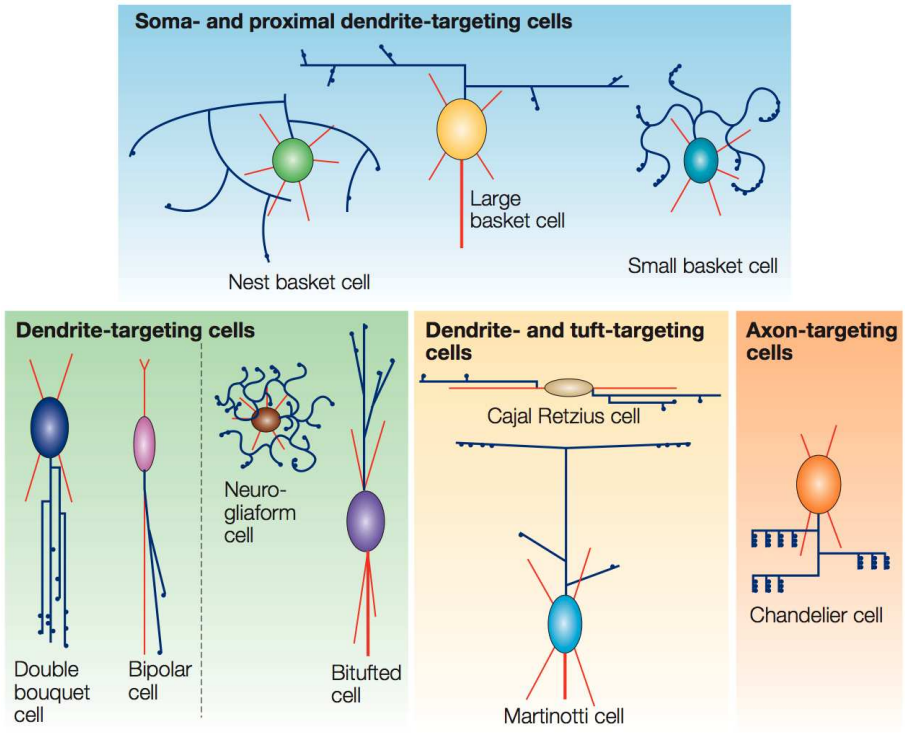

Figure 15: Summary of the main anatomical properties of cortical inhibitory interneurones (dendrites in red and axons in blue) classified according to their preferred axonal targets (From [31]).

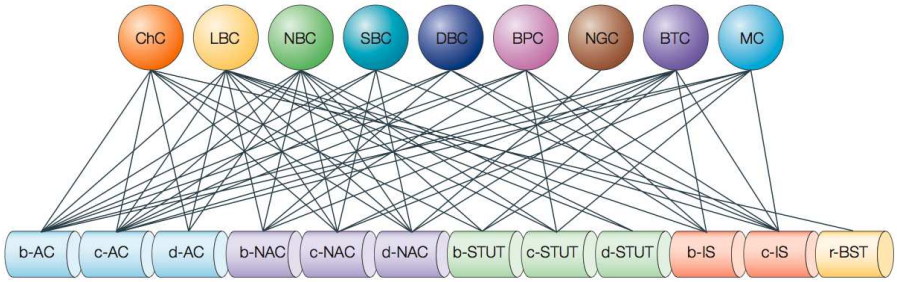

Figure 16: Anatomical-electrophysiological diversity of cortical inhibitory neurons. Above circles: anatomical types (ChC: chandelier cell, LBC: large basket cell, NBC: nest basket cell, SBC: small basket cell, DBC: double bouquet cell, BPC: bipolar cell, NGC: neurogliaform cell, BTC: bitufted cell, MC: Martinotti cell). Bottom cylinders: types of firing responses to somatic current injections (AC: accomodating, NAC: non-accomodating, STUT: stuttering, IS: irregular spiking, BST: bursting) (From [31]).

- Chandelier cells (ChC) are few compared to BCs. One find them in layers II-VI, especially in layer II/III and V. Their local axonal clusters make numerous branchings and primarily target the axon initial segment (axon hillock) of pyramidal cells. ChCs hence influence the action potentials output of PCs by affecting their generation and timing, possibly silencing the firing of spikes.
- Martinotti cells (MC) are very numerous in all layers. They specialize in projecting their axons toward layer I where they inhibit pyramidal dendrites and can extend horizontally to neighbouring or distant columns. Some of them selectively target layer IV. MCs can also innervate proximal dendrites and somata.
- Double bouquet cells (DBC) have a fascicular axonal cylinder that can extend across all layers and make frequent branching. DBCs mainly innervate dendrites of post-synaptic PCs. They are present layers II-V.
- Bitufted cells (BTC) are similar to BPCs and DBCs, but show a wider horizontal axonal span and less extensive vertical projections. They primarily target pyramidal dendrites.
- Bipolar cells (BPC) are relatively sparsely represented in the cortex and have a narrow vertical axonal tree (less than 50μm wide) that crosses all layers. They show low boutons density and mainly target PCs proximal dendrites. They are found in layers II-IV.
- Neurogliaform cells (NGC) have a dense, complex axonal arborization targetting dendrites of post-synaptic cells. There are few such cells in the cortex, and they mainly lie

in layer IV of cortical sensory regions. They connect on PCs dendrites and make dense interconnections with other interneurones via gap junctions (electrical synapses).

- Layer I contains an heterogeneous population of small interneurones with various axonal arborizations and large neurons with horizontal processes confined to layer I, known as Cajal Retzius cells, that are mostly present during development.

Those neuronal types can be separated into two main classes. The first class contains the *proximal targeting cells*, including basket and chandelier cells, that usually show fast spiking firing patterns, project on proximal compartments of PCs and whose dendrites radiate in all directions from the soma.
The second class is constituted from *low threshold spiking (LTS) dendrite-preferring interneurons*, including Martinotti, double bouquet and bitufted cells, that preferentially target dendrites of post-synaptic PCs, usually show low-threshold spiking and bitufted dendritic trees (issuing from the apical and basal poles of the soma).
Although they preferentially target dendrites, bipolar and neurogliaform cells stand apart from these classes. Bipolar cells firing patterns are stuttering and contrary to LTS neurons, they may receive thalamic input. Neurogliaform cells do not display bitufted dendritic arborization and have very specific firing patterns [31, 53].

2.3.2 Interneurones in the microcircuit

Pyramidal cells receive most of their synapses from other PCs. Inhibitory synapses on PCs are less numerous and mostly arise from neighbouring interneurones. For example, only 16% of synapses on PCs in layer II/III are inhibitory and a majority of these arise from interneurones lying in the same cortical column and layer; only 15% of them come from interneurones belonging to an other column.
Nevertheless, the balance between excitation and inhibition in the brain is maintained over a large dynamic range and for many stimuli, suggesting a reliable activation of the inhibitory pathway [32]. This activation is crucial since the lack of inhibition have been linked to various pathologies, including epilepsy [12]. Recent findings have shown that layer V PCs evoke inhibitory responses in neighbouring PCs via the interplay of Martinotti cells [43]. Moreover, the probability for such a *disynaptic inhibition* has been observed to be more than double the probability of direct connections between layer V PCs, suggesting a high connection probability of Martinotti cells on layer V PCs.
Excitatory synapses onto interneurones mostly arise on dendrites and sometimes on somata. Intralaminar connections from spiny excitatory cells to interneurones are frequent and do not appear to show striking preference for any particular interneuronal class. We should also mention interlaminar back projections from PCs of layer III and V (see above). Some classes of inhibitory interneurones even receive direct input from the thalamus, mainly proximal targeting cells and bipolar cells. LTS neurons seem to receive little or no input from the thalamus.
Relatively fewer studies have investigated connections between interneurones, but some of

them indicate that connections between interneurones of all classes are dense and involve quite disparate classes and interneurones in different layers. Basket cells, especially LBCs, seem to have a prominent role in governing the activity of inhibitory networks in the cortex [50]. They make numerous connections with other neighbouring basket cells and also contact dendrite-preferring neurons [31, 53]. Dense networks of interneurones having similar properties, connected through gap junctions (i.e. electrical synapses), have also been observed in the cortex[2] [18]. Cortical electrical synapses seem to be an exclusive property of interneurones since none have yet been found among PCs. They favor high synchronization between neighbouring inhibitory cells of the same class [48, 49].
To finish, we should also mention *shunting inhibition*. GABA-ergic neurons do not only influence their post-synaptic targets by hyperpolarizing the post-synaptic compartment membrane. GABA neurotransmitter exists in two forms: $GABA_A$ and $GABA_B$. $GABA_A$ inhibition is mediated by Cl^- channels and the concentration gradient for Cl^- across the cell membrane determines the nature of the inhibitory effect. If the synaptic reversal potential of the post-synaptic cell is below its resting potential, inhibition will be hyperpolarizing. In contrast, if the synaptic reversal potential is near the resting potential the input resistance is reduced locally and, following Ohm's law, the amplitude of subsequent excitatory postsynaptic potentials can be dramatically reduced. Shunting synapses are more effective if the inhibitory synapse occurs between an excitatory synapse and the action potential initiation site, typically on the perisomatic region of the post-synaptic cell.

3 Connectivity within a cortical area

Now we need to know how connections are distributed horizontally inside a cortical area. Indeed the axonal trees of neurons ramify horizontally and connect to more or less distant post-synaptic targets. Axon collaterals (branches of the axonal tree emerging from the main axon of a neuron) can even make intracortical horizontal projections up to several millimeters. Most inhibitory and excitatory interneurones (spiny stellate cells) make essentially local projections in the same column, while pyramidal cells and large basket cells also form extensive trans-columnar projections. Several studies have discussed the lateral distribution of connections in mammalians visual cortex [56, 55, 46, 25, 26, 24, 11] and the barrel cortex of the rat [1, 28, 41, 8, 40, 17, 15].

3.1 Mammalians visual cortex

The main feature of horizontal connectivity in mammalians primary visual areas (monkeys and cats, but not rats) is the patchy, orientation-preference-biased pattern of pyramidal cells long-range projections (see figure 17 and 18).
In [11], the authors examine the relationship between excitatory lateral connections and orientation maps in the cat primary visual cortex. They show that a good fit is obtained

[2] The probability of gap junction between two neighbouring interneurones of the same class is higher than 50%; this probability becomes inferior to 5% if the neurons belong to different classes.

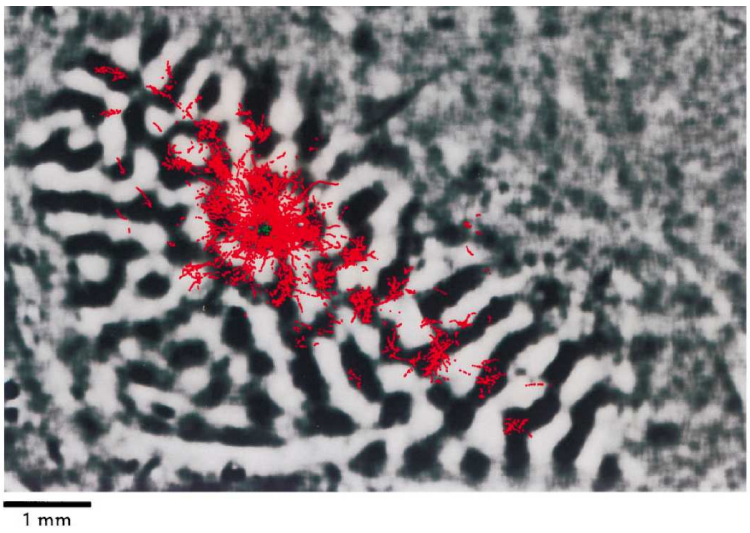

Figure 17: *Reconstruction of axonal boutons distibution (red) from a pool of biocytin-injected pyramidal neurons in layer II/III of the tree shrew visual cortex (green). Strongly marked black and white stripes indicate V1 (black stripes are active areas for a* $90°$ *stimulus). A central patch of radius* $\sim 500\mu m$ *is observed, surrounded by smaller patches extending over several millimeters (From [6]).*

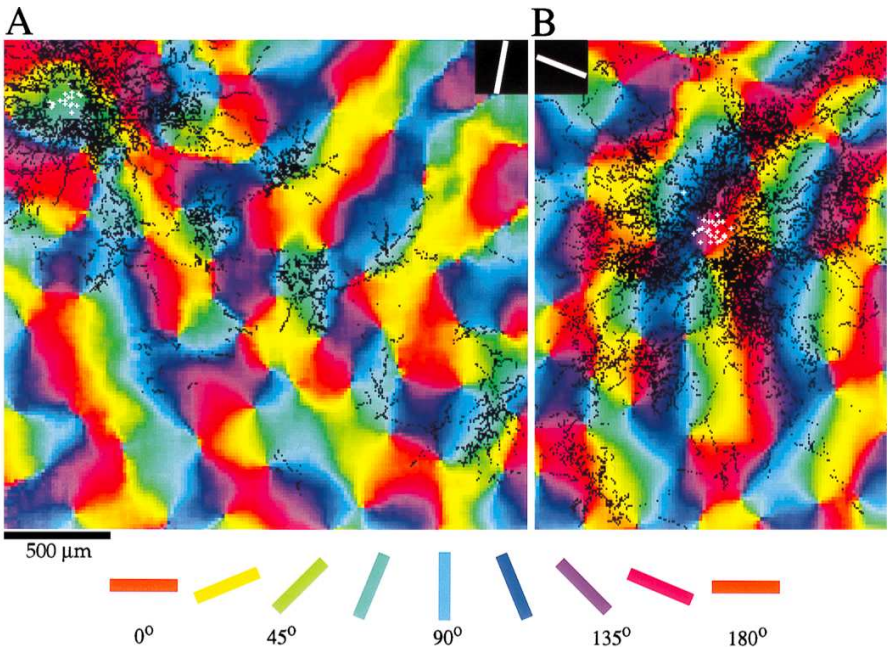

Figure 18: *Reconstruction of axonal boutons distibution (black) from a pool of biocytin-injected pyramidal neurons (white crosses) in layer II/III of the tree shrew visual cortex. A central patch of radius* $\sim 500\mu m$ *is observed, that show no orientation selectivity. Surrounding smaller patches more acutely match areas with similar orientation preference to the preferred orientation of the injection site. The white bar at the corner of each image indicates the preferred orientation of the injected site (From [6]).*

by matching the central connection patch made by layer II/III PCs with non-orientation selective boutons (those that do not specifically target neurons that have the same orientation preference as the biocytin injection site) and the surrounding patches with regions of same orientation preference as the injection site. This means that proximal connections do not discriminate their target in terms of orientation preference while distal patches do (figure 18). This tendency is however not clear when tracking the targets of single cells, whose long-range projections are not necessarily patchy nor orientation selective. This suggests both that this bimodal approximation of PCs lateral connections is consistent with the neural masses approach and that it fails to model the complexity of the precise functional network.
One last striking feature in PCs long-range projections is the observed anisotropy in the patches spatial distribution. It appears that the main direction of this distribution is orthogonal to the preferred orientation of the injected site (see figure 18).

Large basket cells also make trans-columnar projections, up to one or two millimeters, but these are not patchy and have a lesser, 2-3 times smaller spatial extent than projections from excitatory cells (see figure 19) [26].
However, these projections show distance-dependent selectivity according to orientation as well as direction preference. In [25] the authors observe that specificity expresses in two features of the axonal arbour of a large basket cell. The proximal and distal parts of the arbour have distinct orientation and direction selectivities. While local projections show similar preferences to that of the parent soma, distal ones terminate most frequently in regions of non-preferred orientation and opposite preferred direction. Moreover, the axonal arbour of large basket cells can be dissected into two main trees bifurcating near the soma of the cell, which show different orientation selectivity in their distal parts.
So, inhibitory horizontal projections would mainly have two functional roles: distally supressing non-preferred responses, and proximally helping to sharpen orientation and direction tunings because of their looser selectivity compared to excitatory projections.

3.2 Barrel cortex of the rat

The barrel cortex of the rat has a striking columnar organization in layer IV (see figure 7). This layer can be divided into two main regions: the barrels, corresponding to whiskers, and the septum, made of the neurons lying between the barrels. Evidence has been shown that barrel- and septum-related pathways are partially segregated. Barrels and septa receive sensory input from different subcortical pathways, and in layer IV, septum cells preferentially innervate other septum cells while barrel cells mostly innervate neurons in the same barrel. This segregation has been proven to extend to layer II/III by the interplay of layer IV spiny cells projections [8, 23]. We will focus on the connectivity among barrel-related columns, defined by the width of barrels and spanning the whole depth of the cortex.
Layer IV neurons are basically limited to processing information that originates from the associated whisker and form the predominant source of excitatory inputs to layer II/III, that occur on the basal dendrites of PCs in the same column. On the contrary, layer

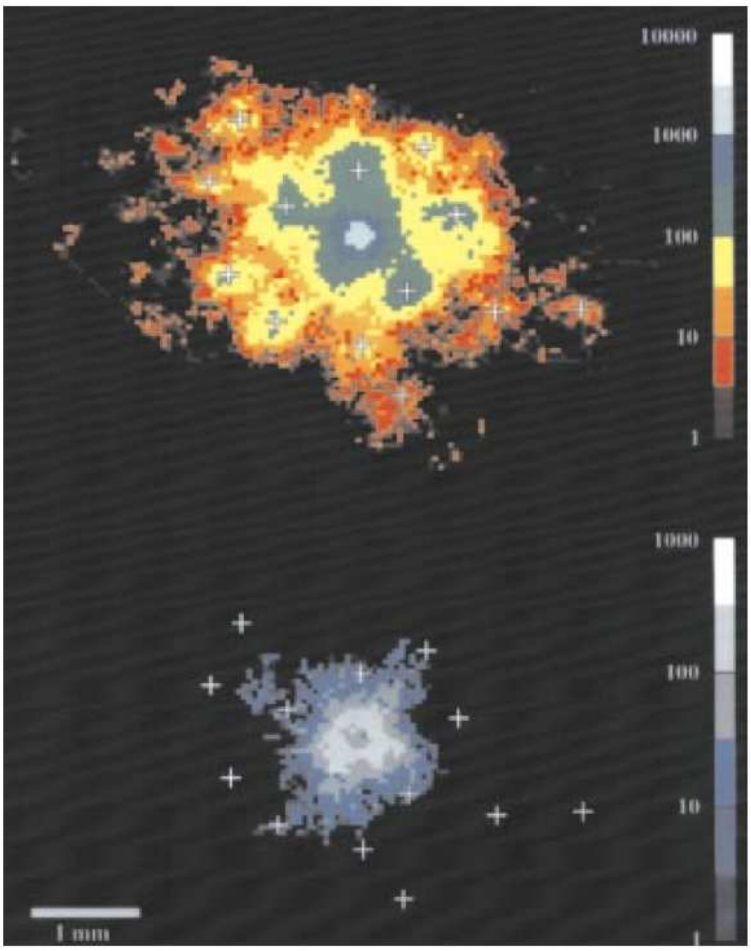

Figure 19: *Comparative distributions of bouton density for pyramidal (up) and basket cells (down). PCs axonal projections form long-range clusters while basket cells ones do not and are less widely spread (From [26]).*

II/III exhibit both intra- and trans-columnar interactions. So, it appears that this circuitry ensures segregation of specific tactile stimuli (in layer IV) as well as transfer of information to neighbouring columns (in layer II/III). In layer II/III, the neuronal activity in the barrel cortex spreads laterally in an oval pattern, preferentially along the rows of the barrel field, as suggested anatomically by the bias of elongation in the direction of barrel rows observed in apical dendrites and axonal arborizations of layer II/III PCs (see figure 20 and 12).

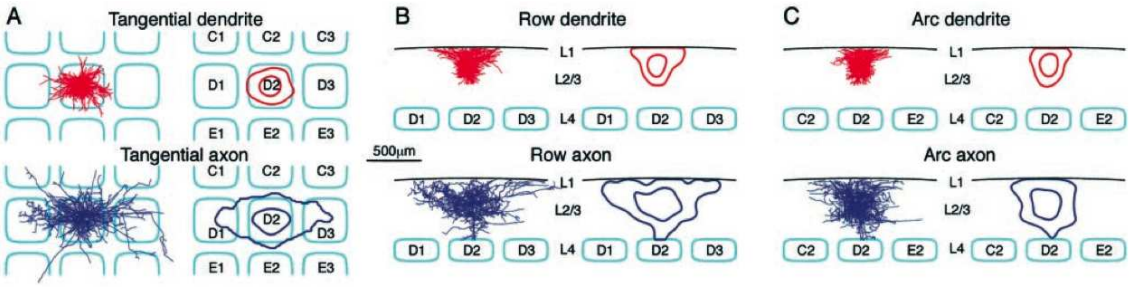

Figure 20: *A. Coronal view of rat barrel cortex (barrels in layer IV are delineated by light blue lines, showing three rows, C, D and E, and three arcs, 1, 2 and 3) showing the preferred elongation of axonal and dendritic trees of layer II/III PCs in the direction of rows. B-C. Corresponding side views (From [39]).*

So, we have seen that horizontal trans-columnar connectivity has an important functional role in the cortex and expresses through different arborization patterns. In the two particular examples we have reviewed, two features must be retained as fundamental: the strong intracolumnar connectivity and the global horizontal decrease of connection intensity with the distance separating neural masses.

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